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Anthocyanin variation in individual 'Shiraz' berries as affected by exposure and position on the rachis

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Summary

This study was done on 'Shiraz'/Richter 99 grapes with the intention to define the variation of skin anthocyanin content in a single berry. The effects of berry position (on the rachis), berry exposure, berry weight category, part of the skin of a single berry, and their interactions, were analysed. The anthocyanin concentration of externally facing berries decreased and that of the internally facing berries increased from the apical part to the basal part of the bunch. Highest values were recorded in internally facing berries and lowest values in the externally facing (exposed) berries, of the basal rachis area. Anthocyanin values of small berries seemed to decrease from the apical part of the rachis to the basal part, whereas those of the larger berries generally increased. Anthocyanin distribution in the skin of a single berry was similar for all the berry weight categories. The median concentric layer of the berry showed the highest anthocyanin concentration. The study showed significant intra-berry variation, indicating that even at full ripeness stage there is still variation in every single smaller or larger berry. The results highlighted the very complicated management challenges to grape producers to increase bunch uniformity in quantity and quality.

K e y w o r d s : *Vitis vinifera,* 'Shiraz', berry variability, berry position, anthocyanin content.

Introduction

Among the grape secondary metabolites, anthocyanins play an essential role in the color of grapes and wine (Jackson and Lombard 1993, Mazza and Francis 1995, Adams 2006, Downey *et al.* 2006, Kennedy *et al.* 2006, Boss and Davies 2009, He *et al.* 2010) and in protection due to their free radical scavenging and antioxidant activity (He *et al.* 2010). Grape skin color is usually determined by the content and composition of anthocyanins in epidermal and hypodermal cells (Kliewer and Torres 1972, Shiraishi and Watanabe 1994). In the cells, they are located inside the vacuoles in a free, non-complexed form (Hrazdina and Moskowitz 1980, Amrani Joutei *et al*. 2005), from where they diffuse into the must and wine during the maceration and fermentation steps of winemaking.

The accumulation of anthocyanin in the berry skin begins with an initial slow rate of accumulation, which is followed by a rapid increase, a stable period, and finally a decline at the end of ripening (Mateus *et al*. 2002, Gholami 2004). The final amount of anthocyanin present in the skin of the grape berry depends on the cultivar (Mazza and Francis 1995), stage of maturity (Hunter *et al*. 2004, Fournand *et al.* 2006, Guidoni *et al.* 2008, Holt *et al.* 2010), terroir, viticulture practices, light exposure, temperature, and water and nitrogen availability (Hunter *et al*. 1991, Mazza and Miniati 1993, Pisciotta *et al*. 2004, Downey *et al*. 2006, Barbagallo *et al*. 2007, He *et al*. 2010, Hunter *et al*. 2010, Ollé *et al.* 2011). Light and temperature appear to have a synergistic effect at moderate temperature and an antagonistic effect at high temperature (Tarara *et al*. 2008). Exposure to solar radiation increases the proportion of di-hydroxylated anthocyanins in comparison to tri-hydroxylated anthocyanins (Keller *et al*. 1998, Spayd *et al*. 2002, Tarara *et al*. 2008, Chorti *et al*. 2010). High temperatures are mainly reported to increase the proportion of acetylated *versus* non-acetylated forms (Spayd *et al.* 2002, Downey *et al*. 2006, Tarara *et al*. 2008). The microclimate in which the berries develop is therefore extremely important. Considering all the factors that may impact on anthocyanin accummulation, the amount of anthocyanins may display a much wider range of variation than that of other qualitative parameters (Wu Dai *et al*. 2011).

The extraction of anthocyanin from berry skins is critical during winemaking (Guidoni and Hunter 2012) and is complicated by the variation in bunch and berry size, which is commonly observed in wine grape vineyards (Barbagallo *et al*. 2011). This variation may result from vineyard heterogeneity, including, but not limited to, differences in soil characteristics, young vine establishment, graft combination, plant material quality, training and pruning practices, node number, shoot number, bunch number, bunch position, and microclimate (Hunter *et al.* 2010). In the vineyard, variation in the colour of berries is commonly and clearly visible in and between wine grape bunches on the same shoot and between shoots, especially when bunches are compact, not well distributed in the canopy, and exposed to different microclimates. This also often leads to individual berries colouring irregularly. Since skin coloration is one of the most important quality factors of wine grapes (Mizuno *et al*. 2006), quite a number of studies have been done on berry skin total anthocyanin variation within bunches (Barbagallo *et al*. 2011, Pisci-

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otta *et al*. 2012, 2010, Matthews & Nuzzo 2007, Tarter and Keuter 2005, Coombe 1992. The focus of these studies has, however, been on colour variation among berries, but not on variation within an individual berry. Many table grape varieties also do not develop adequate berry color in different parts of the skin (*i.e.* 'Crimson Seedless') (Dokoozlian *et al*. 1995, Abd El-Razek *et al.* 2010).

The purpose of this study was to quantify the irregularity of total anthocyanin evolution in the berry skin of 'Shiraz' on an individual basis under field conditions at full ripeness.

Material and Methods

Vineyard: Grapes from a nine-year-old *Vitis vinifera* L. Shiraz' (clone SH1A) vineyard, grafted onto Richter 99 (*Vitis Berlandieri* x *Vitis rupestris*) (clone RY2A), were used. The vineyard is located at the Experimental Farm of ARC Infruitec-Nietvoorbij in Stellenbosch (Western Cape, South Africa). The area is affected by a Mediterranean climate. The vines were spaced 2.75 m × 1.5 m on a Glenrosa soil (Soil Classification Working Group, 1991) with western aspect (26° slope) and trained onto a 7-wire (cordon wire and three sets of movable wires) Lengthened Perold (Vertical Shoot Positioned) Trellising System (Zeeman 1981). Rows were North-South orientated. Vines were micro-sprinkler irrigated at pea size and at véraison stages (12 h @ 32 $L \cdot h^{-1}$). Vines were pruned to two-bud spurs with a spur spacing of approximately 15 cm in a double cordon. Rye was used as cover crop during winter. Normal cultivation practices for the production of healthy grapes were used.

Measurements: One typical bunch per vine was selected from eight vines according to similar exposure (diffused sunlight) conditions and representative of the 0.15 ha of the vineyard (Di Lorenzo *et al*. 2007) for a total of 1.542 kg of grapes. The bunches represented a total of 911 berries. Sampling was done on 13 March 2007 (full ripeness stage) and the berries were first divided according to position on the rachis (apical, median and basal, by dividing the total rachis length into thirds) and then according to the face of the berry (facing internally towards the canopy-interior or externally towards the canopy-exterior). The majority of the externally facing berries were fully exposed to the East, whereas the majority of the internally facing berries were fully shaded in the center of the canopy. The wings of the bunch were included in a berry position according to the length of the main rachis. Harvested grapes were cooled to the same temperature (20 °C) before processing. Each one of the berries was analyzed in terms of weight (g). Each berry was detached by cutting its pedicel.

After measuring, the skin was cut to open the berry. Three skin disks of 4.67 mm in diameter each were punched from three concentric layers of the berry (apical, median, basal) (Figure). The three skin disks for each concentric layer were then immersed in 2 mL of a methanolic 0.1 % HCL solution at pH 1.0. Samples were stored overnight in the dark, after which they were vortexed without discarding the skins. The absorbance of the solution was immediately read at 520 nm with a LKB Ultrospec IIE UV/VIS spectrophotometer. The surface area of the skin disks per cuvette was calculated as $(r^2*\pi)*3$. The $mg \cdot L^{-1}$ of total anthocyanin was calculated as follows: Abs(520)*17.65 (standard malvidin coefficient as determined on the specific instrument) and then converted in total anthocyanin expressed as $mg \cdot cm^{-2}$. Finally, a total of 7434 skin disks (2478 per berry concentric layer) were processed, after berries showing visual skin damage and those that were too small for disk selection, were rejected.

Figure: Berry skin sampling points (**A** = apical, **M** = median, **B** = basal) for different berry weight categories (1, 2, 3, 4).

After all berries were processed, four different berry classes were selected according to the recorded berry weight of each berry. The four berry classes were as follows: 1 (≤ 1.0 g); 2 (between 1.01 g and 1.50 g); 3 (between 1.51 g and 2.0 g) and 4 (> 2.0 g).

Statistics: Mean, number of cases and variation coefficient are reported. Rachis portion (apical, median, basal), berry exposure (external and internal), part of the berry skin (apical, median, basal) and berry weight categories (1, 2, 3, 4) were considered as factors. A multi-factorial ANOVA was used to test for significant differences in skin color and the existence of interactions between factors [$\alpha = 0.05$, indicated the presence of a statistically significant difference, and $\alpha = 0.01$ was considered highly significant. For significant main effect and their interactions, mean statistical differences were determined using the Tukey's HSD test ($\alpha = 0.05$)].

Each berry was treated as an experimental unit. Statistically significant results are reported. The SYSTAT version 12© program was used to perform the statistical analyses.

Results and Discussion

Tab. 1 shows that there is no evidence of three-way interactions of Portion (P) × Berry exposure (E) × Concentric layer (CL); Portion (P) × Berry exposure (E) × Weight category (WC); Portion (P) × Concentric layer (CL) x Weight category (WC); Berry exposure (E) × Concentric layer (CL) x Weight category (WC); and of the four-way interaction P x E x CL x WC. For the two way interactions P x E; P x CL; P x WC; E x CL; E x WC; CL x WC, only the interaction between P and E, P and WC, and CL and WC are significant, meaning that the effect of the position of the berry on the rachis on the skin color depends on the berry exposure and weight category, and the effect of the concentric layer of the berry in which the color was deter-

Table 1

Effect of the factors berry position, berry exposure; skin concentric layer; and berry weight category and their interactions on the berry skin color expressed as mg·cm^{-2}. *, ** (grey evidenced), and n.s. indicate significance at α = 0.05, at α = 0.01, and not significant, respectively

	F	P	Significance
Source			
Portion (P)	3.576	0.028	*
Berry exposure (E)	2.700	0.100	n.s.
Concentric layer (CL)	32.809	0.000	**
Berry weight category (WC)	6.083	0.000	**
Interaction			
P x E	4.013	0.018	*
P x CL	1.773	0.132	n.s.
P x WC	2.935	0.007	**
E x CL	2.159	0.116	n.s.
E x WC	1.355	0.255	n.s.
CL x WC	4.142	0.000	**
P x E x CL	0.382	0.822	n.s.
P x E x WC	0.996	0.426	n.s.
P x CL x WC	1.230	0.256	n.s.
E x CL x WC	0.259	0.956	n.s.
P x E x CL x WC	0.716	0.737	n.s.

Table 2

Mean, number of cases and coefficient of variation of berry skin anthocyanin (mg·cm^{-2}), sampled from different bunch positions and different exposure

Portion[y]		Berry exposure	
		External[z]	Internal
Apical	mean	0.132 a[y] A[z]	0.126 b B
	n	454	606
	c.v.	24.6	32.7
Median	mean	0.128 ab	0.130 a n.s.
	n	377	579
	c.v.	29.4	26.6
Basal	mean	0.123 b B	0.136 a A
	n	204	258
	c.v.	28.9	18.1

[y] Means within a column followed by a different small letter are significantly different at α = 0.05 (HSD Tukey's test).
[z] Means within corresponding lines followed by a different capital letter are significantly different at α = 0.05 (HSD Tukey's test).
n.s.= non significant.

mined, depends on the berry size. Since there is evidence of two-way interactions, the main effects, namely the position of the berry along the rachis, the berry concentric layer and the berry weight category, are not discussed.

For externally facing berries, the anthocyanin concentration decreased from the apical part of the bunch to the basal (0.132, 0.128 and 0.123 mg·cm^{-2}, respectively, for the apical, median and basal parts), whereas that of the internally facing berries increased (0.126, 0.130 and 0.136 mg·cm^{-2}, respectively, for the apical, median and basal parts). The highest value with the lowest coefficient of variation (18.1 %) was recorded in the internally facing berries of the basal rachis area (0.136 mg·cm^{-2}), while the lowest value was measured in the externally facing berries of the basal rachis area (Tab. 2). It is possible that the basal parts of the bunches were too exposed and the intensity (and composition) of the radiation as well as the temperature may have increased to values beyond that required for optimal anthocyanin evolution.

The berry categories showed different behaviour along the rachis in terms of anthocyanin accumulation. Anthocyanin values of small berries (1st category berry size) seemed to decrease from the apical part of the rachis to the basal part (0.145, 0.129 and 0.112 mg·cm^{-2}, respectively, for the apical, median and basal parts), whereas for the 3rd and 4th category berry size values generally increased. No clear effect was recorded for the 2nd berry size category (Tab. 3). The anthocyanin distribution in the skin of a single berry

Table 3

Mean, number of cases and coefficient of variation of berry skin anthocyanin (mg·cm^{-2}) sampled from different bunch positions and classified by four weight categories

Portion of bunch	Berry weight category			
	1 (≤ 1.0 g)	2 (1.01 - 1.50 g)	3 (1.51 - 2.0 g)	4 (> 2.0 g)
Apical				
mean	0.145 a[y] A[z]	0.132 A	0.123 b B	0.133 A
n	39	404	438	179
c.v.	12.2	25.3	34.2	27.7
Median				
mean	0.129 ab AB	0.134 A	0.126 ab B	0.129 AB
n	33	315	404	204
c.v.	18.9	23.5	30.7	28.6
Basal				
mean	0.112 b B	0.130 n.s. A	0.131 a A	0.135 n.s. A
n	21	195	177	69
c.v.	32.7	23.4	23.6	20.1

[y] Means within a column followed by a different small letter are significantly different at α = 0.05 (HSD Tukey's test).
[z] Means within corresponding lines followed by a different capital letter are significantly different at α = 0.05 (HSD Tukey's test).
n.s. = non-significant.

Table 4

Mean, number of cases and coefficient of variation of berry skin anthocyanin (mg·cm^{-2}) of four berry weight categories and measured in different skin concentric layers

Concentric layer	Berry weight categories											
	1 (≤ 1.0 g)			2 (1.01 - 1.50 g)			3 (1.51 - 2.0 g)			4 (> 2.0 g)		
Apical												
mean	0.112	b[y]	B[z]	0.120	c	B	0.117	c	B	0.128	b	A
n	31			305			340			151		
c.v.	32.4			32.4			36.8			29.0		
Median												
mean	0.143	a	A	0.142	a	A	0.135	a	B	0.141	a	A
n	31			305			340			151		
c.v.	8.2			15.1			23.1			17.6		
Basal												
mean	0.135	a	A	0.136	b	A	0.124	b	B	0.123	b	B
n	31			304			339			150		
c.v.	23.1			22.1			32			32.4		

[y] Means within a column followed by a different small letter are significantly different at α = 0.05 (HSD Tukey's test)
[z] Means within corresponding lines followed by a different capital letter are significantly different at α = 0.05 (HSD Tukey's test)
n.s.= non significant

Table 5

Mean, number of cases and coefficient of variation of berry skin anthocyanin (mg·cm^{-2}) measured in different skin concentric layers

	Concentric layer					
	Apical		Median		Basal	
mean	0.120	c	0.139	a	0.129	b
n	827		827		824	
c.v.	33.7		18.7		28.7	

Means followed by a different small letter are significantly different at α = 0.05 (HSD Tukey's test).

was similar for all the berry weight categories (Tab. 4). The median concentric layer of the berry showed the highest anthocyanin concentration (Tab. 5) and the lowest coefficient of variation in all berry weight categories, compared to the other areas. The equatorial anthocyanin concentration was similar in all berry weight categories. The concentration in the apical part was lower than that of the basal part for all the category sizes, except for the largest berries where the values in the apical part were higher than those in the basal part. The anthocyanin concentration in the apical part of the berry increased from small to large berries (Tab. 4). This is probably related to a different berry development from flowering to ripeness stage (Pisciotta *et al*. 2012). Contrasting results were found in the basal part of the berry, values decreasing from small to large berries.

A different distribution pattern in terms of the mechanical properties (break energy) of the skins of single berries was also found for 'Cabernet Sauvignon', 'Pinot Noir' and 'Nebbiolo' (Letaief *et al*. 2008). However, in the latter study the apical part of the berry had the lowest skin break force and the median part of the berry the highest. A lower skin break force pre-supposes a softer skin and perhaps higher degradation and permeability of the cell membranes. Since the skin disks in this study were of the same diameter, a variation in skin thickness most likely occurred from the apical to the basal part of the berry. This may have led to more or less extraction during the allocated time. Following this, the apical part of the berry should have led to higher extraction and therefore higher values of anthocyanin. This was, however, not found in the 'Shiraz' berries used in this study. A thinner skin in the apical part *versus* the other parts may be a more likely explanation for this finding. It is also possible that the anthocyanin vacuolar inclusions per square cm as explained by Mizuno *et al.* (2006) may have been less in the apical part of the berry, therefore leading to lower anthocyanin contents in this part of the berry.

Conclusions

The study was focused on defining the variation in skin anthocyanin content in a single berry, under the influence of position on the rachis and bunch microclimate in the canopy. Berry exposure, berry weight category, skin partitioning of a single berry and positioning of the berry on the rachis showed clear trends, indicating a very complex anthocyanin distribution pattern and impact of microclimate. The results indicated that even at full ripeness, significant intra-berry variation occurred for every single small or large berry of the bunch. The results pose complicated management challenges to grape producers to increase bunch and berry uniformity quantitatively and qualitatively.

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